

09/554835
410 F 55d PCT/PTO 19 MAY 2000

DIARRHEA
Use of the E.Coli Strain DSM 6601 for Treating Diarrhoea in Veterinary Medicine

The invention relates to the use of Escherichia coli strain DSM 6601 for prevention and treatment of diarrheas caused in animals by microbes.

Diarrhea in humans or animals is understood as frequent discharge of fluid feces for various causes, such as an allergic reaction to certain foods, but it suggests a reaction to microorganisms, especially bacteria, fungi or viruses or the toxins thereof. Because of great water and salt loss, prolonged diarrheas can lead to serious complications and even death. Severe diarrheas therefore always require treatment, especially in the case of young or already weakened humans or animals.

In a large proportion of cases, diarrheas are caused by bacteria that do not belong to the normal microorganisms of the flora of the affected body. Certain pathogenic strains of Escherichia coli, Salmonella and Shigella in particular play a major role in this regard. Diarrheas can be caused not only by bacteria, however, but also by infections with viruses, especially coronaviruses and rotaviruses, or by fungi, which in most cases are members of the Eumycota. Whereas diarrhea caused by bacteria can usually be combated very effectively now by administration of sorbents or nonsystemic sulfonamides or antibiotics, pharmaceuticals which can be used successfully in virus infections have not yet

- 2 -

been available, and hardly any pharmaceuticals are effective against colonization of the gastrointestinal tract with fungi.

Fungi are defined as a polyphylogenetic group of chlorophyll-free, heterotrophic and eukaryotic organisms, which can be single-celled or multiple-celled and whose reproduction proceeds with mitosis and meiosis, as for all eukaryotes, and by the formation of sexual or asexual spores or even by budding. The single-celled fungi that propagate only by budding are frequently grouped together as yeasts, even though the yeasts represent a conglomerate of different classes. Almost all Eumycota, or in other words the true fungi, pass through two or more morphologically distinguishable stages during their individual development, namely as teleomorphs, in which the spores are formed after meiosis, and one or more stages as anamorphs, in which spore formation is not associated with meiosis. Fungi whose teleomorphs are unknown or which have lost the ability to form such are grouped together as Fungi imperfecti. Exact botanical classification is still unresolved for many of the fungi.

Because of their lack of chlorophyll, all fungi obtain their nutrition heterotrophically by degradation of organic substances; there are therefore saprophytes. In medicine a distinction is made between the opportunists, or in other words saprophytes which can become pathogenic rarely and only under strictly defined conditions. They include, for example, *Candida* and *Aspergillus*. Pathogenic saprophytes are exogenous organisms which live a normal saprophytic existence, but they must be regarded as pathogenic for humans or animals under all circumstances if an infection has developed. The fungi also

- 3 -

include obligate parasites, which cannot vegetate outside the host organism and which are found only in humans or animals; they include most dermatophytes.

One substantial difference between bacteria and fungi is based on the fact that fungi, in contrast to bacteria, are eukaryotes, meaning they possess a nucleus and membrane and, again in contrast to bacteria, they also possess mitochondria; another on the fact that the cell wall consists of chitin and/or cellulose, whereas the cell wall of the bacteria is formed from mucopeptides. This also explains why most antibiotics which are effective for bacteria fail for fungi, since in many known antibiotics the bacterial wall or the mitochondria are the main points of attack of these agents.

Some antibiotics are also known which can also be used for systemic and not merely topical treatment of fungal infections and which are characterized by a polyene structure, such as amphotericin B, griseofulvin, natamycin and nystatin. Synthetic systemic antimycotics are flucytosin and a series of azole derivatives such as ketoconazole and miconazole or fluconazole and itraconazole.

Whereas the exact mechanism of action of the polyene antibiotics is not yet known in all details, the synthetic azole derivatives act on the ergosterol synthesis in the cell membrane and thus influence the permeability of the cell wall.

- 4 -

A disadvantage of all antimycotics known heretofore, even those which can be used systemically, is that they usually act not as fungicides but merely as fungistats, thus necessitating a relatively long treatment period. Furthermore, cross resistances develop, both to the polyene antibiotics and to the azole compounds. In addition, finally, the price of these products is relatively high, thus hindering broad application in veterinary medicine.

There therefore continues to be a further need for veterinary pharmaceuticals which are capable effectively of combating diarrheas caused in animals substantially pathogenic fungi or even by the co-involvement thereof.

Completely surprisingly, it has now been discovered that such diarrheas can be effectively combated by using *Escherichia coli* strain DSM 6601, even when otherwise conventional medication with compounds having activity toward fungi has failed.

Escherichia coli, abbreviated hereinafter as *E. coli*, exists in numerous varieties, which differ as regards capsule antigens, surface antigens and flagella antigens and can therefore be subdivided into numerous serological types. Classification by serotypes, however, does not provide any indication of the different virulence of the pathogens. Representatives of one and the same serotype can have different pathogenic potential both in the human and in the animal body, ranging in the extreme case from avirulent to highly pathogenic. It is known, however, that *E. coli* strain DSM 6601 is rated as nonpathogenic to humans or animals. As an example, this strain is used in human medicine as a substitution preparation in infectious intestinal diseases due to *Salmonella* or *Shigella*, both in acute and

chronic cases. This *E. coli* strain is also used successfully in substitution therapy for other disorders of the intestinal flora, for example after antibiotic treatment or irradiation. It has not been unequivocally clarified whether what actually happens is that this special *E. coli* strain merely displaces the pathogenic bacteria strains, including the corresponding variants of *E. coli* or *Proteus*, thus causing a reduction in the toxins, or whether the metabolic products of *E. coli* strain DSM 6601 have a therapeutic effect in their own right.

Starting from these known explanations of the mechanism of action of living *E. coli* microorganisms, however, it could in no way be anticipated that treatment with these living cultures would have surprisingly extensive efficacy toward infection of the intestinal tract of animals with fungi, and in this context especially yeasts. Certainly apathogenic *E. coli* strains were occasionally in veterinary medicine in the fifties for diseases of cattle or pigs, sometimes also involving diarrheas, but the objective was therapy for nutritional disorders in piglets (Fischer, W., Experiences of a practicing veterinarian in the treatment of sick piglets from 1945 to 1950; Dissertation, Munich University 1950), or the treatment of Semper disease in cattle, which according to the results of this publication was obviously based on deficient nutrition due to the geology of the region (Häfele W., "Semper disease", a nutritional and developmental disorder of cattle in the Upper Black Forest in the vicinity of St. Blasien; Dissertation, Vet. Med. Animal Clinic, Munich University 1952). These early attempts to use certain *coli* strains experimentally for special diseases of pigs or cattle were entirely isolated, and not once did they provide the motivation to undertake further experiments of this type in diarrheas caused by bacteria. All the more surprising was the discovery that *E. coli* strain DSM 6601 exhibits a surprising effect even in

- 6 -

intestinal diseases caused exclusively or substantially by fungi, since fungal infections are particularly difficult to combat, especially when the mucous membranes of the intestine are affected, since the preparations used for bacterial infections have practically zero effect.

Heretofore surprisingly little has been known about the normal intestinal flora in various animal species, but infestation of the gastrointestinal tract with fungi and especially with yeasts must always be regarded as a pathological event.

The surprisingly rapid efficacy of the treatment with *E. coli* strain DSM 6601 suggests that the effect of this treatment does not depend or does not depend only on substitution of the fungal flora by a healthy bacterial flora, but instead that the strain contributes largely to an increase in the body's endogenous defense mechanisms, presumably because the metabolic products of this strain have a considerable immuno-stimulating effect.

The invention will be explained in more detail hereinafter by means of an example:

Example:

In a dairy farm in Saxony, which possessed on average a stock of 1480 milk cows, 600 heifers and young cows as well as 185 calves, the calf stock was characterized in early January 1997 by very good rearing results and low morbidity and mortality. Manifest gastric and intestinal diseases with diarrhea were extremely infrequent. Losses of calves were always below the 3% limit relative to the number of live-born calves.

By late January 1997 there was observed a sudden increase in the incidence of diarrheas among the stock, with clinically pronounced symptoms of gastroenteritis. The diseases began among the calves aged from seven to eleven days, were of various duration and were characterized by a morbidity of more than 90% and a mortality of more than 10%. The course of this disease was marked by the following symptoms: initially greenish-yellowish diarrhea with the consistency of thick paste, after which, in the further course of the disease, the feces became slimy, increasingly thin and mostly watery. The body temperature readings were slightly elevated at the beginning of the disease (39.7 to 39.9 °C), but then dropped rapidly in the further course and reached only still lower limits, some in the range of 37.8 to 37.5 °C. The diseased calves exhibited pronounced aversion to suckling and increasing weakness, ultimately just lying down in one place in the further course of the disease. Seriously sick animals had to be forcibly fed.

After an illness duration of three to nine days, the losses became increasingly worse. The autopsies performed consistently revealed the most prominent

findings, namely a fluid, flocculent to slightly bloody intestinal content and advanced, ulcerative inflammations of the omasum and abomasum. In the month of April 1997, the incidence of the disease reached values of almost 100% and the mortality rose to more than 15%.

The microbiological studies of the gastric and intestinal contents and of the internal organs were initially without specific results until February 1997, when yeasts of the genus *Candida* (*C. glabrata* and *C. albicans*) were isolated from the gastric and intestinal contents of two dead calves. Intestinally pathogenic *E. coli* were not found. These yeasts were detected in all further autopsies of dead calves and in the studies of fecal specimens of calves and their mothers. Further specific studies of fecal and colostric specimens of the cows and heifers yielded identical results. Supplementary studies for viruses, namely coronaviruses and rotaviruses, as well as for cryptosporidae revealed coronavirus particles only in two cases and rotaviruses in one case among dead calves.

The origin of the yeasts remained inexplicable for some time, until March 1997, when a study of brewery residues used as fodder was able to detect yeasts (with *Candida glabrata* dominating) in a concentration of 3.6×10^7 CFU per g of fodder.

The therapeutic measures immediately performed extensively, such as dietary drinks, antiphlogistics, astringents, electrolytes and cardiovascular agents led only to unsatisfactory results. When sulfonamides and antibiotics were used, the animals died six to eight hours after application. Once these therapies had proved unsuccessful, therapeutics containing humic acids as the active agents

were used. Even these actions did not achieve any perceptible improvements of the occurrence of the disease.

Of the 236 calves born alive in two months from 24 April to 23 June 1997, all animals became sick and 41 died in this period, corresponding to a proportion of 17.4%. Of those, 29 animals, corresponding to 70.7% of the losses, died from diarrheas; eight animals, corresponding to 19.5% of the losses, died from diarrheas with concomitant bronchopneumonia; and four animals, corresponding to 9.8% of the losses, died from other causes.

In the period from 25 June 1997 to 04 September 1997, a suspension of living *E. coli* strain DSM 6601 was then administered to 300 newborn calves in an amount of 15.0 ml per calf per day – corresponding to ... CFU/ml. This dose was independent of the weight and age of the animal and was administered orally, specifically for a duration of 10 to 13 days after birth. In the period from 24 June to 08 July 1997, 61 calves were treated in this way. Two exhibited diarrhea, corresponding to a morbidity of 3.3%. None of the animals died of this disease.

In the period from 09 July to 23 July 1997, 49 calves were treated correspondingly. None of these suffered from gastroenteritis with diarrhea. The mortality due to this disease was therefore 0%.

- 10 -

In the period from 23 July to 06 August 1997, 64 calves were treated. Two exhibited diarrhea, corresponding to a morbidity of 3.1%. None of the animals died of this disease, and so for this period the mortality was equal to 0.

After all existing therapeutic options had been exhausted, the occurrence of diarrheas due to gastroenteritides in suckling calves was prevented almost completely by the preventive and therapeutic application of a suspension of *E. coli* strain DSM 6601.

It was possible to considerably reduce the use of other pharmaceuticals and dietetics, and so the costs expended for these substances were lowered by 70%.